LOCALIZATION AND REACTIONS OF A PHEROMONE DEGRADATIVE ENZYME ISOLATED FROM AN INSECT ANTENNA*

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Abstract. A fluid isolated from chemosensory sensilla on the antennae of males and females of the cabbage looper, *Trichoplusia ni* (Hübner), was fractionated by gel filtration. Enzymatic degradation of the pheromone was associated with 2 distinct groups of fractions. The first group contained membrane fragments, vesicles, and membrane-bound enzyme or insoluble enzyme aggregates. The second group of fractions contained solublilized enzyme. Because of the method of isolating the 'sensillum liquor' by breaking the tips of the hairs, membranes from chemoreceptor cell dendrites should greatly predominate. In general, 3 isomers and the saturated analog of the pheromone were hydrolyzed more rapidly than the pheromone.

1. Introduction

Enzymatic and protein-binding reactions of proteins isolated from antennal sensilla of the adult male cabbage looper, *Trichoplusia ni* (Hübner), and its pheromone [(*Z*)–7–dodecen–1–ol acetate] have been observed and speculatively implicated in transduction of olfaction (Ferkovich *et al.*, 1973a, b). Other examples of such enzymatic action or protein binding of specific sex pheromones have been demonstrated in *Bombyx mori* (L.) (Kasang and Kaissling, 1972; Kasang, 1973, 1974) and in *Antheraea pernyi* Güerin-Meneville (Riddiford, 1970). Also, similar observations of selective antennal protein binding by anti-feeding compounds were observed with *Periplaneta americana* (L.) (Norris *et al.*, 1970; Ferkovich and Norris, 1972).

The response spectrum of the cabbage looper to its pheromone and to various isomers and analogs of the pheromone is relatively specific and has been studied behaviorally (Jacobson *et al.*, 1968; Berger and Canerday, 1968; Toba *et al.*, 1970; and Kaae *et al.*, 1973) and electrophysiologically (Grant, 1970; Payne *et al.*, 1970; Gaston *et al.*, 1972; and Mayer, 1973, and unpublished). Therefore, we reasoned that the sensillar enzyme(s), if actually involved in olfactory transduction, would show some degree of substrate specificity that could be correlated with behavioral responses.

This report describes in detail the initial steps used to purify and localize the enzyme system reported earlier (Ferkovich *et al.*, 1973a, b) and some preliminary reactions and observations thereon.

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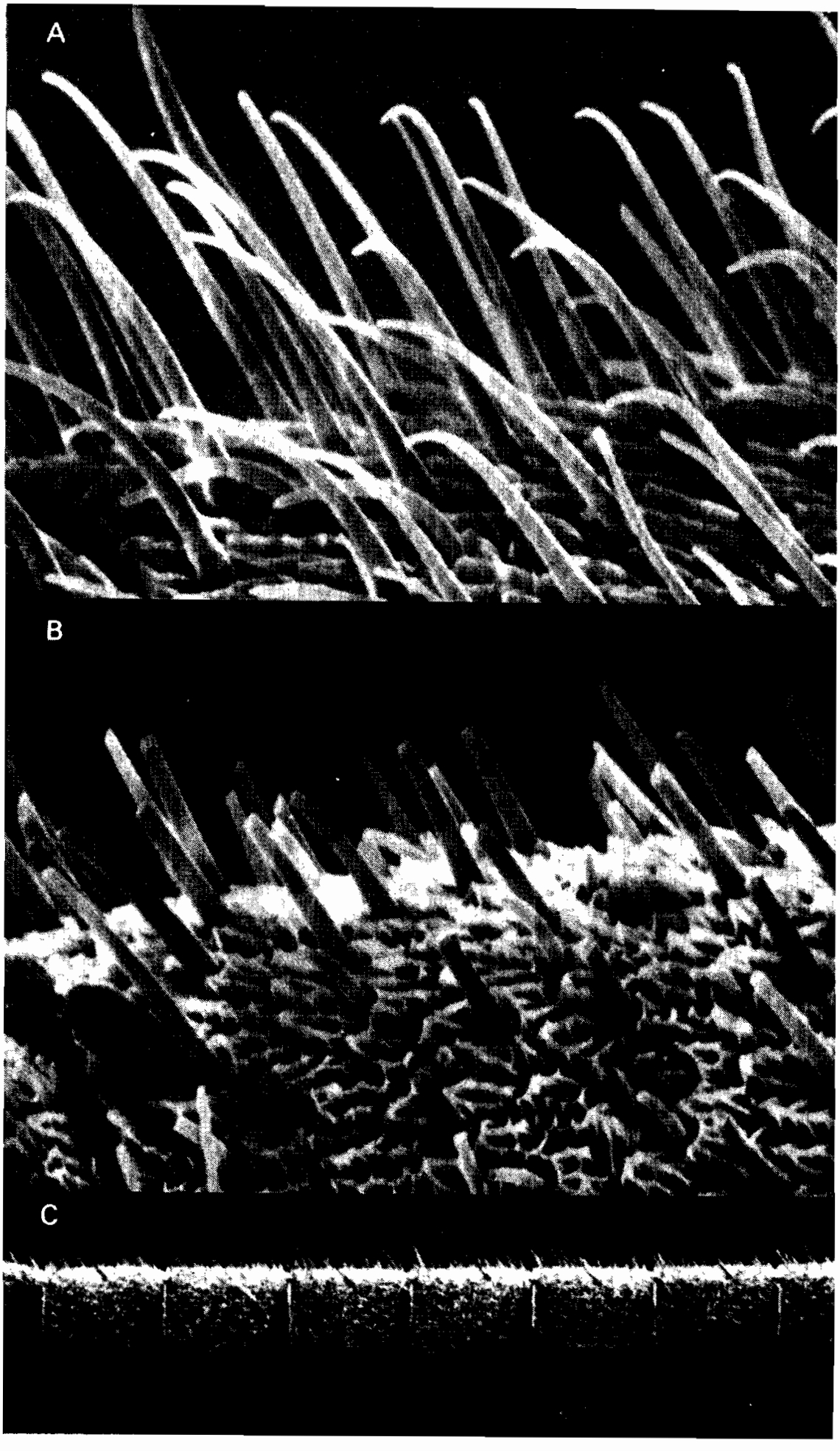
A
B
C

2. Materials and Methods

2.1. Preparation and gel filtration of antennal proteins

All excisions of antennae and legs from laboratory-reared, 3–5-day-old adult moths were done at 4 °C in 0.5 *M* sucrose buffered with 0.05 *M* Tris-HCl, pH 7.5. Generally, 500 pairs of male antennae (yielding about 0.38 mg protein), 650 pairs of female antennae (yielding about 0.2 mg protein) and 350 pairs of male legs (yielding about 0.1 mg protein) were used for each gel filtration separation. As described previously (Ferkovich *et al.*, 1973b) the antennae or legs were excised into a 5-ml beaker in ice containing 1.0 ml of buffer and sonicated in an ice bath in a Ladd, Model T-586 (82 kHz) ultrasonic cleaner at full power for 10–30 min depending on the tissue. The yield of protein obtained depended on the length of sonication, but more importantly on the number of pairs of organs sonicated. The best sonication procedure was to successively sonicate in one beaker less than 125 pairs of antennae at a time. The sonicated remainder of the bodies of the antennae were saved, rinsed, and the rinse combined with the sonicate. As can be observed in Figure 1, only the tips of the chemoreceptor sensilla were fractured. The combined sonicate and rinse solutions were centrifuged at 47 000 g for 10 min to remove scales and broken sensilla tips, and the protein content was determined by the method of Lowry *et al.* (1951).

The resultant supernatant (approx. 1.5 ml) was applied to a column (0.9 cm × 60 cm) containing Sephadex G-200 equilibrated with 0.05 *M* Tris-HCl, pH 7.5. The column was operated at 4 °C with a flow rate of 5 ml hr^{-1} and the effluent was collected in 0.42 ml fractions. In another experiment, the fluid released from sonicated antennae was sonicated a second time in buffer to which Triton X-100 (0.6%) was added. For this sonication a Biosonic III was used at maximum intensity (approx. 1 W cm^{-2} of the microtip probe). The sonicate was then applied to a Sephadex G-200 column equilibrated with 0.6% Triton X-100 in 0.05 *M* Tris-HCl, pH 7.5.

The elution patterns of the protein preparations were continuously monitored at 280 nm. The apparent molecular weight of the Triton X-100 solubilized enzyme was determined with the following protein standards in 0.6% Triton X-100 in buffer: aldolase (1.58×10^5 M.W.), ovalbumin (4.5×10^4 M.W.), chymotrypsinogen A (2.5×10^4 M.W.), and ribonuclease A (1.37×10^4 M.W.) (Granath and Kvist, 1967).

2.2. Electron microscopy

Electron microscopy was used to examine the material in the first peak eluting off a Sephadex column G-200. The fluid released by the sonication of 600 pairs of male antennae was applied to the column after prior centrifugation. The fractions of the void volume peak were combined and then centrifuged at 100.000 g for 2 hr to sedi-

Fig. 1. Demonstrates the initial purity of chemoreceptive elements obtained by sonication: (A) chemoreceptive sensilla *inter alia* of a typical flagellal segment of *T. ni* antenna prior to sonication, X2100; (B) appearance of sensilla after 5-10 min sonication X2100; (C) demonstrates that the antenna remains intact except for the fractured sensilla (X140).

ment any particulate material. The resultant pellet was then prepared for examination in an electron microscope according to Ferkovich *et al.* (1974).

2.3. Enzyme assay

Either 0.25 or 0.5 ml of individual protein fractions or combined fractions was pipetted into 0.8 ml incubation vials. A sonicated water suspension of pheromone, or its isomers (100 μg 0.1 ml^{-1}) was prepared as before (Ferkovich *et al.*, 1973b). One-hundred μl of this suspension was mixed with the antenna or leg protein in a final volume of 0.6 ml and incubated for either 1, 18 or 24 hr at 22°C. The reaction was terminated by mixing 0.1 ml of diethyl ether with the contents of the incubation vial. After phase separation the relative amounts of pheromone remaining and of alcohol [(*Z*)–7–dodecen–1–ol] produced was measured by gas liquid chromatography (GLC) as described previously (Ferkovich *et al.*, 1973a, b).

2.4. Treatment of male antennal sonicate with nucleases and enzyme inhibitors

Nucleases – A 0.5 ml aliquot of combined and separate fractions obtained from gel filtration of the antennal sonicate was incubated with 5 mg of DNAse 1 plus 0.06% $MgSO_4$ (bovine pancreas, B grade) or RNAse (bovine pancreas, A grade) (both obtained from Calbiochem) in 1.6 ml of 0.05 *M* Tris-HCl, pH 7.5 at 37°C for 30 min. Five-tenths ml of buffer was substituted for the protein fraction as a control. The pheromone hydrolyzing activity of the samples was then determined by GLC.

Phospholipase C – The antennal sonicate was collected and prepared for gel filtration chromatography and was incubated with phospholipase C (Type I, *Cl. welchii* from Sigma Chemical Co.) at 1:1, antennal protein: phospholipase C at pH 7.5, for 30 min at 37°C prior to gel filtration (Finean and Coleman, 1970).

DFP and Eserine – The sonicate (34 μg protein) was incubated with diisopropyl phosphorofluoridate and eserine (3×10^{-2} *M*) in separate runs for 15 min at 37°C, pH 7.5 and then applied to a column of Sephadex G-200 equilibrated with 1×10^{-3} *M* of the appropriate inhibitor in buffer.

Chemicals – The synthetic pheromone was 95+% pure; the saturated pheromone analog, dodecan–1–ol acetate, and the (*E*)–7–dodecen–1–ol acetate were 98+% pure; and the pheromone isomers, (*Z*)–8– and (*Z*)–9–dodecen–1–ol acetates, were 95+% pure by GLC analysis.

3. Results

3.1. Gel filtration of enzyme

The elution pattern of male antennal protein and the enzymatic activity of individual fractions are shown in Figure 2. Two major absorption peaks were resolved and two peaks of enzyme activity were observed. One peak of enzyme coincided with the absorbance peak near the void volume (peak A) and the other (peak B) coincided with a shoulder preceding the slower-eluting absorption peak. The UV-absorbing material eluting after 35 ml never degraded the pheromone and contained no protein. A similar

absorbance and enzyme activity pattern was observed with female antennal and male leg sonicates.

Examination by transmission electron microscopy of the material in the first absorption peak eluting off the Sephadex column revealed fragments of membranes and vesicles (Figure 3).

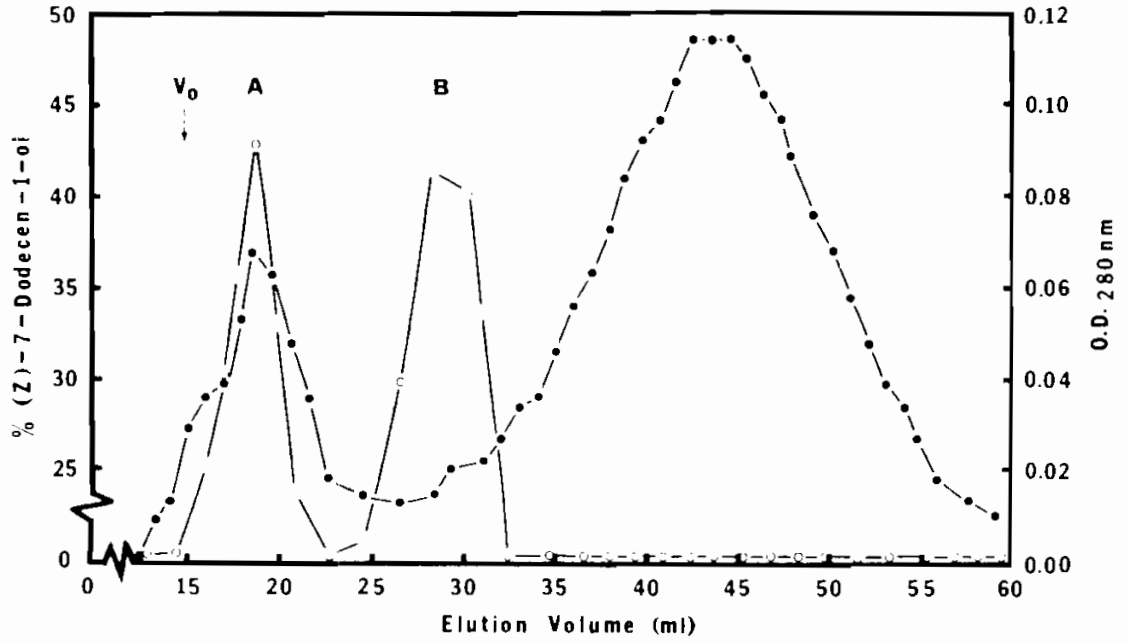

Fig. 2. Elution pattern of male antennal sonicate (0.2 mg protein) on a column of Sephadex G-200 with Tris-HCl, pH 7.5 as eluant and an elution rate of 11.4 ml hr^{-1}. Percentage product [(*Z*)-7-dodecen-1-ol] after 12 hr incubation at 22 °C with 100 μg of pheromone per each 0.95 ml fraction collected (○, left ordinate) absorbance at 280 nm (●, right ordinate).

3.2. Effects of phospholipase C and Triton X-100 on the elution pattern of enzyme

The incubation with phospholipase C of a separate, freshly isolated sample of antennal sonicate prior to gel filtration chromatography resulted in a decrease in the enzymatic activity associated with peak A and a concomitant increase in the enzymatic activity of peak B. There was no indication that the total enzymatic activity of the sample was changed by this treatment.

The elution pattern of Triton X-100 solubilized male antennal protein and enzymatic activity of individual fractions is shown in Figure 4. Only one peak of enzyme activity was obtained, indicating that the addition of Triton X-100 either disrupted protein aggregates or solubilized a membrane-bound enzyme. The apparent molecular weight of this Triton X-100 solubilized enzyme was 38 000.

3.3. Effect of nucleases and inhibitors on enzyme activity

The 280/260 nm absorbance ratio of enzyme peaks A and B from male antennae was

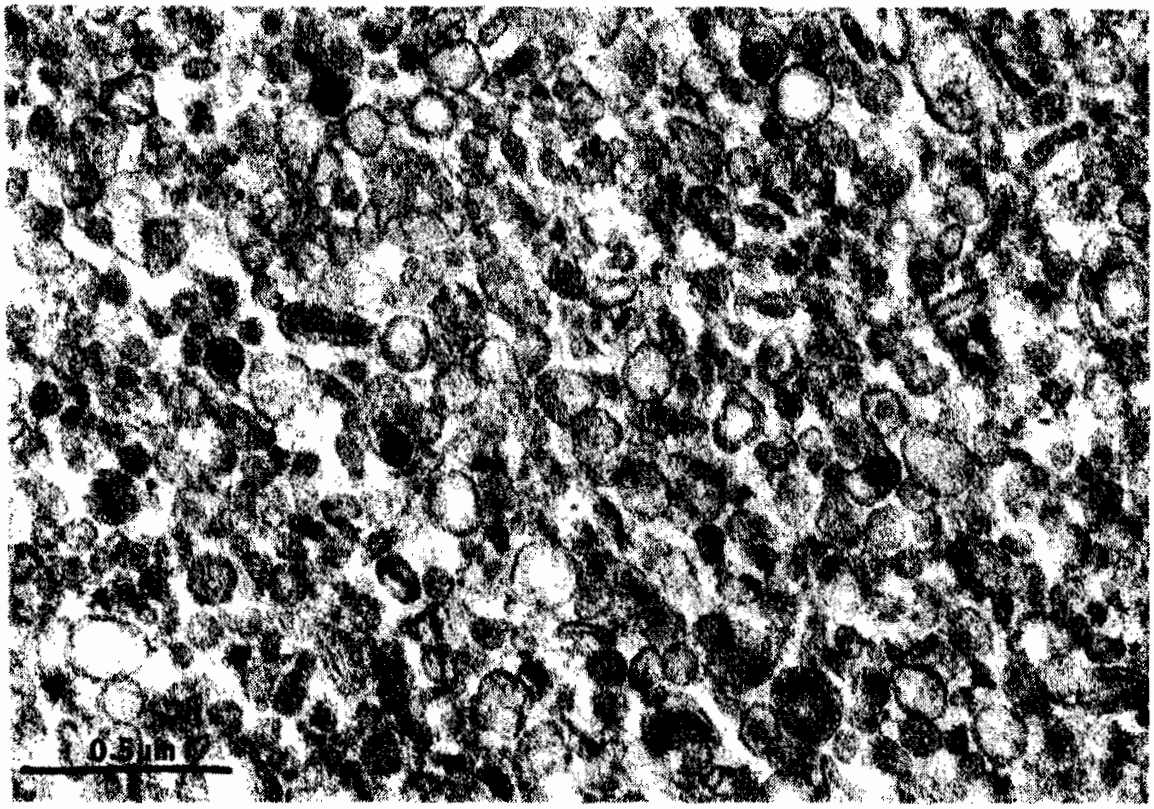

Fig. 3. Electron micrograph of membrane vesicles eluting in the void volume of a gel permeation separation of male antenal sonicate (X61 500).

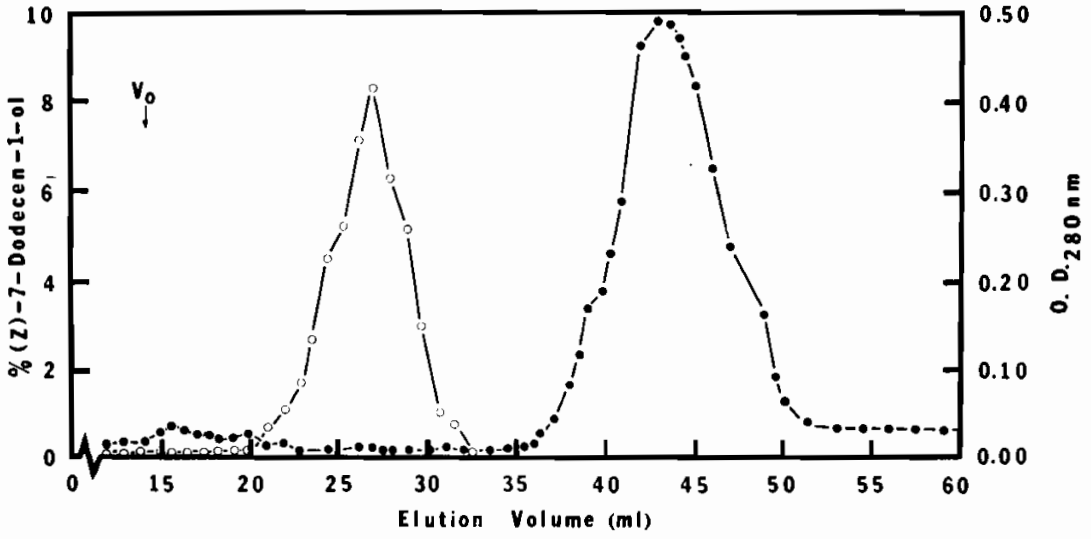

Fig. 4. Elution pattern of Triton X-100 solubilized male antennal sonicate (1.9 mg protein) on a column of Sephadex G-200 with 0.6% Triton X-100 in 0.05 M Tris-HCl, pH 7.5 as eluant and an elution rate of 5.32 ml hr^{-1}. Percentage product [(*Z*)-7-dodecen-1-ol] after 60 min incubation at 22 °C with 100 µg of pheromone per each 0.44 ml fraction collected (○, left ordinate); absorbance at 280 nm (●, right ordinate).

0.455 and 0.320, respectively. This low ratio suggested that either nucleic acids or other materials absorbing predominantly at 260 nm were associated with the enzyme. Treatment of the enzyme in both peaks with DNAse and RNAse, however, did not influence the rate of pheromone hydrolysis.

Short term incubation of male antennal sonicate with the two esterase inhibitors, DFP and eserine, did not inhibit activity of the enzyme peak A or B, and thus suggested that the enzyme(s) was not a general, nonspecific esterase.

3.4. Relative hydrolysis of pheromone and isomers in vitro

The results of tests of enzyme specificity with male and female antennae and male legs are summarized in Table I. In this experiment fractions containing the first (A) and second (B) peaks of enzyme activity eluting from the column were combined and aliquots were incubated with the various chemicals. The most interesting aspect in this rough measure of specificity is that almost all chemicals were degraded to the alcohol to a greater extent than was the pheromone and that no great differences were observed between enzyme in peak A and B.

TABLE I

Percentage isomeric alcohol produced after 18 hr incubation with combined fractions containing enzyme peak A (15–22 ml eluant) or with peak B (25–32 ml eluant) from male and female antennae and male legs

Chemical	Percentage conversion of acetate to isomeric alcohol[a] by:					
	Male antenna Enzyme peak		Female antenna Enzyme peak		Male legs Enzyme peak	
	A[b]	B[d]	A[b]	B[d]	A[c]	B[d]
(*Z*)-7-dodecen-1-ol acetate	51.5	56.9	54.6	45.8	5.3	27.5
(*Z*)-8-dodecen-1-ol acetate	99.2	92.9	97.6	89.5	25.3	55.8
(*Z*)-9-dodecen-1-ol acetate	39.0	74.7	59.6	40.7	1.6	8.7
(*E*)-7-dodecen-1-ol acetate	90.9	31.4	69.4	–	4.2	21.4
Dodecan-1-ol acetate	60.8	49.2	34.7	25.8	3.1	11.7

[a] (*Z*)-7-dodecen-1-ol was confirmed as the reaction product from (*Z*)-7-dodecen-1-ol acetate by comparing retention times on two GLC columns and by mass spectrometry.

[b] 11 μg and [c] 3 μg protein, 100 μg pheromone 0.2 ml^{-1} 0.05 *M* Tris-HCl, pH 7.5 at 22 °C for 60 min.

[d] No protein detected by the method of Lowry *et al.* (1951).

A second experiment was performed in which only the male antennal enzymes were used. This experiment was designed to duplicate the previous experiment in part and to establish whether individual column fractions representing the maxima of the peaks of enzymatic activity reacted differently than combined fractions of these peaks. The results (Table II) again confirmed that the pheromone was generally degraded less than any of the other isomers. No major differences were observed between indi-

TABLE II

Percentage isomeric alcohol produced after 1- and 24-hr incubation with individual and combined fractions of enzyme in peaks A and B from male antennae

Enzyme peak	Fractions		Incubation time (hr)		Percentage conversion of dodecen-1-ol acetate to isomeric alcohol			
	Combined	Individual	1	24	(*Z*)-7-	(*E*)-7-	(*Z*)-8-	(*Z*)-9-
A	+		+		5.8	12.1	51.7	23.2
	+			+	68.1	99.4	100.0	97.4
		+	+		1.1	5.1	20.5	9.3
		+		+	8.8	20.2	79.7	55.3
B	+		+		32.5	20.5	54.9	21.4
	+			+	96.6	83.0	100.0	47.7
		+	+		4.4	6.1	59.1	35.2
		+		+	14.7	22.0	96.9	78.7

vidual and grouped column fractions in peaks A and B. Possibly, the decreased enzymatic hydrolysis of the pheromone could be the result of nonenzymatic binding to receptors or to the absence of a cofactor.

These *in vitro* data are in essential agreement with those of Kasang and Kaissling (1972) and Kasang (1973) who found that 2 analogs of bombykol were metabolized in or on the sensilla of *B. mori in vivo* to the same extent as bombykol, the pheromone. Kinetic studies of the enzyme system *in vitro*, which are now underway, will be necessary to firmly establish enzyme specificity and to correlate this specificity with that *in vivo*.

4. Discussion

This report provides details of a technique that results in a rapid isolation of elements of chemoreceptors and which eliminates most of the extraneous cellular elements generally obtained in homogenates. Some nonchemoreceptor contaminants escape from the cut ends of the antennae during sonication and various types of chemoreceptors may be fractured. However, the moth antenna has large numbers of pheromone-receptor cells and it is reasonable to assume that reactions of this receptor type with pheromone is responsible in large part for the reactions measured here. The females of *T. ni* also perceive their own pheromone (Grant, 1970; Mayer, unpublished) and we obtain identical biochemical reactions. At this time we do not comprehend the biochemical relationships of the legs to pheromone hydrolysis; however, no evidence is available concerning the presence of olfactory chemoreceptors on the legs of moths, although there are some trichoid sensilla present.

The evidence presented here, and that obtained previously (Ferkovich *et al.*, 1973a, b), lead us to the assumption that we are working with at least a part of the olfactory transductory process. The site of this process, as we conceive it, is the dendritic membrane, contiguous chemically or physically with the external cuticular surface. More-

over, the cuticular surface may also have a physico/chemical role in transport of the odor molecule to the pore (Adam and Delbrück, 1968) and/or in degradation of excess pheromone (Mayer, 1975). We do not infer that the transductory mechanism is primarily an enzymatic process (Dravnieks, 1966); we do believe, though, that the protein binding we observe (Ferkovich *et al.*, 1973a) is an integral part of the mechanism. The enzymatic cleavage of the pheromone may occur after the event of transduction.

The following evidence provided in this report strongly intimates that some of the pheromone-degrading enzyme is membrane-bound: (1) sonication with a detergent (Triton X-100) eliminated enzymatic activity associated with membrane elements; and (2) incubation with phospholipase C which degrades membranes (Finean and Coleman, 1970) shifted the elution of enzymatic activity away from the void volume (Vo) of the column toward regions where the solubilized enzyme would elute. When these two observations are considered in conjunction with the sonication technique (Ferkovich *et al.*, 1973b) which predominantly breaks off only the chemoreceptive sensilla, the evidence becomes strongly indicative that at least some of the enzyme(s) is located in or on the dendritic endings of the primary olfactory receptor cell. However, another possibility is that the enzyme may be located on the cuticular surface (Mayer, 1975) and that it was dissociated from the cuticle during sonication.

Mayer (1975) demonstrated substrate specificity *in vivo* of the antennal enzyme(s) for the pheromone over closely related isomers and analogs at short exposure times (4 s). With longer periods of exposure (> 1 min) of the pheromone to the antennae, no pattern of specificity was observed. In the *in vitro* studies reported here, however, the antennal enzymes degraded the pheromone more slowly than the behaviorally inactive isomers of the pheromone. The enzymatic activity was not monitored at short time intervals as in the *in vivo* studies, and it is possible that the *in vitro* enzyme specificity would be similar to that in the short term *in vivo* studies. Another possibility is that the difference in the *in vitro* and *in vivo* incubations was due to nonenzymatic binding by protein 'acceptors' (Kaissling, 1969) such is as observed in hormone receptor binding (Freedman, 1974).

The net effect of such acceptors preferentially binding the pheromone would result in a greater decrease in the rate of enzyme hydrolysis of the pheromone than on the isomers of the pheromone. This later supposition is supported by our previous UV absorption studies which showed that a nonenzymatic binding also occurs with another odorant molecule [(*Z*)-7-dodecen-1-ol].

These and other observations reinforce our inclination to analogize these reactions of olfactory cells to the acetylcholine receptor-acetylcholinesterase system of other nerve cells and synapse mechanisms (Kitz, 1973).

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References

Adam, G. and Delbrück, M.: 1968, 'Reduction of Dimensionality in Biological Diffusion Processes', in A. Rich and N. Davidson (eds.), *Structural Chemistry and Molecular Biology*, pp. 198–215, Freeman Co., San Francisco.

Berger, R. S. and Canerday, T. D.: 1968, 'Specificity of the Cabbage Looper Sex Attractant', *J. Econ. Ent.* **61**, 452–454.

Dravnieks, A.: 1966, 'Current Status of Odor Theories', *Adv. Chem. Ser.* **56**, 29–52.

Ferkovich, S. M. and Norris, D. M.: 1972, 'Antennal Proteins Involved in the Quinone Inhibition of Insect Feeding', *Experientia* **28**, 978.

Ferkovich, S. M., Mayer, M. S., and Rutter, R. R.: 1973a, 'Conversion of the Sex Pheromone of the Cabbage Looper', *Nature* **242**, 53–55.

Ferkovich, S. M., Mayer, M. S., and Rutter, R. R.: 1973b, 'Sex Pheromone of the Cabbage Looper: Reactions with Antennal Proteins *in vitro*', *J. Insect Physiol.* **19**, 2231–2243.

Ferkovich, S. M., Rutter, R. R., and Anthony, D. W.: 1974, 'Spectrophotometric Measurement of Juvenile Hormone Binding in Subcellular Components of the Indian Meal Moth', *J. Insect Physiol.* **20**, 1943–1948.

Finean, J. B. and Coleman, R.: 1970, 'Integration of Structural and Biochemical Approaches in the Study of Cell Membranes', in J. R. Villanuva and F. Ponz (eds.), *Membrane Structure and Function*, pp. 9–16.

Freedman, R. B.: 1974, 'Hormone/Membrane Interactions as Models for Chemoreceptor Phenomena', in T. M. Poynder (ed.), *Transduction Mechanisms in Chemoreception*, pp. 115–123, Information Retrieval Ltd., London.

Gaston, L. K., Payne, T. L., Takahashi, S., and Shorey, H. H.: 1972, 'Correlation of Chemical Structure and Sex Pheromone. Activity in *Trichoplusia ni* (Noctuidae)', in D. Schneider (ed.), *IV Intern. Sym. Olfaction and Taste IV*, pp. 167–173, Wissenschaftliche Verlagsgesellschaft MBH, Stuttgart.

Granath, K. A. and Kvist, B. E.: 1967, 'Molecular Weight Distribution Analysis by Gel Chromatography on Sephadex', *J. Chromatogr.* **28**, 69–81.

Grant, G. G.: 1970, *Electrophysiological Studies on the Cabbage Looper: Electroantennogram Responses to the Female Pheromone and Hair-Pencil Scent and Anatomy of Their Glandular Sources.* Ph. D. Dissertation, Virginia Polytechnic Institute and State University, Virginia.

Jacobson, M., Toba, H. H., Debolt, J., and Kishaba, A. N.: 1968, 'Insect Sex Attractants. VIII: Structure-Activity Relationships in Sex Attractant for Male Cabbage Loopers', *J. Econ. Ent.* **61**, 84–85.

Kaae, R. S., Shorey, H. H., McFarland, S. O., and Gaston, L. K.: 1973, 'Sex Pheromones of Lepidoptera. XXXVII: Role of Sex Pheromones and Other Factors in Reproductive Isolation Among Ten Species of Noctuidae', *Ann. Ent. Soc. Am.* **66**, 444–448.

Kaissling, K.-E.: 1969, 'Kinetics of Olfactory Receptor Potentials', in C. Pfaffmann (ed.), *Olfaction and Taste, III*, pp. 52–70.

Kasang, G.: 1973, 'Physikochemische Vorgänge beim Riechen des Seidenspinners', *Naturwiss.* **60**, 95–101.

Kasang, G.: 1974, 'Uptake of the Sex Pheromone ^{3}H-Bombykol and Related Compounds by Male and Female *Bombyx* Antennae', *J. Insect Physiol.* **20**, 2407–2422.

Kasang, G. and Kaissling, K.-E.: 1972, 'Specificity of Primary and Secondary Olfactory Processes in *Bombyx* Antennae', in D. Schneider (ed.), *IV Intern. Sym. Olfaction and Taste*, pp. 200–206, Wissenschaftliche Verlagsgesellschaft MBH, Stuttgart.

Kitz, R. J.: 1973, 'Molecular Pharmacology of Acetylcholinesterase', in R. M. Featherstone (ed.), *A Guide to Molecular Pharmacology-Toxicology, Part I*, pp. 333–374, Marcel Dekker, Inc., New York.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: 1951, 'Protein Measurement with Folin Phenol Reagent', *J. Biol. Chem.* **193**, 267–275.

Mayer, M. S.: 1973, 'Electrophysiological Correlates of Attraction in *Trichoplusia ni*', *J. Insect Physiol.* **19**, 1191–1198.

Mayer, M. S.: 1975, 'Hydrolysis of Pheromone by the Antenna of *Trichoplusia ni* (Hübner)', *Experientia* **31**, 452–454.

Norris, D. M.: 1969, 'Transduction Mechanism in Olfaction and Gustation', *Nature* **222**, 1263–1264.

Norris, D. M., Ferkovich, S. M., Baker, J. E., Rozenthal, I. M., and Borg, T. K.: 1970, 'Energy Transduction: Inhibition of Cockroach Feeding by Naphthoquinone', *Science* **170**, 754–755.

Payne, T. C., Shorey, H. H., and Gaston, L. K.: 1970, 'Sex Pheromones of Noctuid Moths: Factors Influencing Antennal Responsiveness in Males of *Trichoplusia ni*', *J. Insect Physiol.* **16**, 1043–1055.

Riddiford, L. M.: 1970, 'Antennal Proteins of Saturniid Moths – their Possible Role in Olfaction', *J. Insect Physiol.* **16**, 653–660.

Toba, H. H., Green, N., Kishaba, A. N., Jacobson, M., and Deboli, J. W.: 1970, 'Response of Male Cabbage Loopers to 15 Isomers and Congeners of the Looper Pheromone', *J. Econ. Ent.* **63**, 1048–1051.